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Nanomedicine Opened New Horizons for Metal Nanoparticles to Treat Multi-Drug Resistant Organisms

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A B S T R A C T

Novel studies and technologies are devoted to understanding the mechanisms of disease for the design of new drugs, but unfortunately infectious diseases continue to be a major health burden world wide**.** Multi-drug resistance still growing problem in the treatment of infectious diseases, and the widespread use of broad-spectrum antibiotics has produced antibiotic resistance for many human bacterial pathogens. Advances in nanotechnology have opened new horizons in nanomedicine, allowing the synthesis of nanoparticles that can be assembled into complex architectures. Since ancient times, metal was known for its anti-bacterial effects and for centuries it has been used for prevention and control of disparate infections. Currently nanotechnology and nanomaterials are fully integrated in common applications and objects that we use every day. In addition, the metal nanoparticles are attracting much interest because of their potent and wide spectrum antibacterial activity. Bacterial strains that are resistant to current antibiotics have become serious public health problems that increase the need to develop new bactericidal materials. Therefore, nanoparticles have gained importance in the field of biomedicine.

Introduction

Drug-resistant bacteria are emerging pathogens whose resistance profiles present a major hallenge for containing their spread and their impact on human healt (1,6). Increasing hospital and community-acquired infections due to bacterial multidrug-resistant (MDR) pathogens for which current antibiotic therapies are not effective represent a growing problem. Antimicrobial resistance is one of the major threats to human health (1), since it determines an increase of morbidity and mortality as a consequence of the most common bacterial diseases (2).Resistance genes have recently emerged favoured by improper use of antibiotics(3, 4)hence, the first step in combating resistance envisions the reduction of antibiotic consumption (5). Antimicrobial resistance is a complex mechanism whose etiology depends on the individual, the bacterial strains and resistance mechanisms that are developed (6). The emergence of resistance against newly developed antibiotics further supports the need for

innovation, monitoring of antibiotic consumption, prevention, diagnosis and rapid reduction in the misuse of these drugs (7). It is necessary to optimize antibiotics' pharmacokinetics and pharmacodynamics in order to improve treatment outcomes and reduce the toxicity and the risk of developing resistance (8).

To address the problem of resistance, it will be necessary to change the protocols of use of antimicrobials so that these drugs are administered only when all other treatment options have failed (4), and joint efforts of governments and academic networks are needed to fight against the globally spreading of multidrug resistant pathogens. Today, there is aneed to seek alternative treatments (9). Non-traditional antibacterial agents are thus of great interest to overcome resistance that develops from several pathogenic microorganisms against most of the commonly used antibiotics (4). Nanotechnology offers unique approaches to control a wide variety of biological and medical processes that occur at nanometer length, and it is believed to have a successful impact on biology and medicine.(8,9)

Nanoparticles and Multi-drug Resistance Organisms

Nanoparticles are being viewed as elementary building blocks of nanotechnology, nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance (10).In recent years, the use of nanoparticles, particularly metal nanoparticles has expanded in biomedical research. They are used in bioimaging, delivery, drug delivery and other diagnotic and therapeutics applications, due to their unique properties of small size, large surface area to volume ratio, high reactivity to the living cells, stability over high temperatures and translocation into the cells, etc(9,11,13). Metal has always been used against various diseases; in the past it found use as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria (14, 16, 22) due to its low cytotoxicity (17). Silver ions have been shown to interact with the thiol group in enzymes and in active them leading to cell death, they have also shown to interact with DNA to enhance pyrimidine dimerization by the photodynamic reaction and possibly prevent DNA replication. Elevated levels of metals ions inside a cell cause oxidative stress and the generation of hydrogen peroxide, causing oxidative damage to cells, decline in the membrane integrity of microbes, leading to leakage of specific essential cell nutrients, this leads to desiccation and subsequent cell death. Also nanoparticles can bind to protin, and this binding leads to loss-of-function of the protein, and/or breakdown of the protein into nonfunctional portions (19,86).NPs were considered, in recent years, particularly attractive for the production of a new class of antimicrobials (4,18,25) opening up a completely new way to combat a wide range of bacterial pathogens. Although the highly antibacterial effect of NPs has been widely described, their mechanism of action is yet to be fully elucidated. In fact, the potent antibacterial and broad-spectrum activity against morphologically and metabolically different microorganisms seems to be correlated with multifaceted mechanism by which nanoparticles interact with microbes (8).Moreover, their particular structure and the different modes of establishing an interaction with bacterial surfaces may offer a unique and under probed antibacterial mechanism to exploit.

From a structural point of view, NPs have at least one dimension in different size and

shapes range from 1 to 100 nm and more importantly, as particle size decreases, the surface area-to-volume ratio greatly increases.(22,35) As a consequence, the physical, chemical and biological properties are markedly different from those of the bulk material of origin. Several mechanisms of action of NPs and antibiofilms activities have been proposed by different authors, and the most corroborated are described below (4,19).

Drug Mechanisms of Action on Bacteria

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are often categorized according to their principal mechanism of action. Mechanisms include interference with cell wall synthesis (eg, beta-lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of ametabolic pathway (trimethoprim. sulfamethoxazole) and disruption of bacterial membrane structure (polymyxins and daptomycin)(3,8). Almost as soon as antibacterial drugs were deployed, bacteria respond by manifesting various forms of resistance. As antimicrobial usage increase, so did the level and of resistance mechanisms exhibited by bacterial pathogens, bacteria may manifest resistance to antibacterial drugs through a variety of mechanisms (8). Some species such as *Acinetobacter baumannii* becomes resistant to antibiotics by alteration of cell wall and cytoplasm. (26, 27) *Escherichia coli* Alteration of membrane permeability and respiration (26,2,44) *Enterococcus faecalis* Alteration of cell wall and cytoplasm (42,45,46). *Klebsiella pneumoniae* Alteration of membrane (28,41,47), *Listeria monocytogenes* Morphological changes, separation of the cytoplasmic membrane from the cell wall, plasmolysis (47), *Micrococcus luteus* Alteration of membrane (28), Nitrifying bacteria inhibits respiratory activity (31), *Pseudomonas aeruginosa* Irreversible damage on bacterial cells; Alteration of membrane permeability and respiration (17,36,48,50), *Proteus mirabilis* Alteration of cell wall and cytoplasm. (43) *Staphylococcus aureus* Irreversible damage on bacterial cells (31,37,39,41). *Staphylococcus epidermidis* Inhibition of bacterial DNA replication, bacterial cytoplasm membranes damage, modification of intracellular ATP levels (36,52) *Salmonella typhi* Inhibition of bacterial DNA replication, bacterial cytoplasm membranes damage and modification of intracellular ATP levels (48,51) and *Vibrio cholerae* Alteration of membrane permeability and respiration (33).

NPs are able to physically interact with the cell surface of various bacteria, this is particularly important in the case of Gram-negative bacteria where numerous studies have observed the adhesion and accumulation of NPs to the bacterial surface. Many studies have reported that NPs can damage cell membranes leading to structural changes, which render bacteria more permeable (14,53). This effect is highly influenced by the nanoparticles' size, shape and concentration (53,56) and a study using *Escherichia coli* confirmed that NPs accumulation on the membrane cell creates gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death (14,19). Several studies have shown that AgNP activity is strongly dependent on the size (46,47). Infact, the bactericidal activity of NPs of smaller dimensions (<30 nm) was found to be optimal against *Staphylococcus aureus* and *Klebsiella pneumoniae* (49). Smaller

nanoparticles seem to have a superior ability to penetrate into bacteria. In fact, the interactions with the membranes and any resulting damage, which may lead to cell death, are certainly more evident in the case of nanoparticles with smaller diameter and a positive zeta potential. Electrostatic forces that develop when nanoparticles with a positive zeta potential encounter bacteria with a negative surface charge promote a closer attraction and interaction between the two entities and possibly the penetration in bacterial membranes(32). Indeed, the zeta potential along with the size of the nanoparticles is a fundamental parameter for controlling the antimicrobial activity and more effective nanoparticles have a positive zeta potential and a reduced size. As said earlier, NPs have a surface/volume ratio much greater than the corresponding bulk material; therefore, modalities and amount of the interactions with the bacterial surfaces are facilitated and determine a higher antibacterial activity(34).

One should also consider that a certain amount of cationic metal is released from the nanoparticles when these are dissolved in water or when they penetrate into the cells. In effect, nanoparticles have a higher antibacterial activity than the free ions of metal, whereby the antibacterial properties are attributed to both the physical properties of nanoparticles and the elution of metal ions (57). It is likely that a combined effect between the activity of the nanoparticles and free ions contributes in different ways to produce a strong antibacterial activity of broad spectrum(8,10). Furthermore, the fact that bacterial resistance to elemental metal is extremely rare (58), emphasizes the presence of multiple bactericidal mechanisms that act in synergy. The metal ions bind to the protein and nucleic acid negatively charged, causing structural changes and deformations in the wall, in the membranes and in the nucleic acids of the bacterial cell. In fact, metal ions interact with a number of electron donor functional groups such as thiols, phosphates, hydroxyls, imidazoles and indoles (10,12). The NPs also damage membranes and induce the release of reactive oxygen species (ROS), forming free radicals with a powerful bactericidal action (46). Metal ions or small NPs can easily enter the microbial body causing the damage of its intracellular structures. As a consequence ribosomes may be denatured with inhibition of protein synthesis, as well as translation and transcription can be blocked by the binding with the genetic material of the bacterial cell (33, 59, 60). Protein synthesis has been shown to be altered by treatment with NPs and proteomic data have shown an accumulation of immature precursors of membrane proteins resulting in destabilization of the composition of the outer membrane (61), in Figure 1, we summarize the possible toxicity mechanisms of NPs.

The correlation between the bactericidal effect and AgNP concentrations is bacterial class dependent (22). Indeed, *Pseudomonas aeruginosa* and *Vibrio cholera* were more resistant than *E. coli* and *Salmonella typhi*, but at concentrations above 75 μg/mL, the bacterial growth was completely abolished (50). In this perspective, Kim *et al.*,2007(34) studied NPs antimicrobial activity against *E. coli* and *S. aureus* showing that *E. coli* was inhibited at low concentrations, while the inhibitory effects on the growth of *S. aureus* were less marked (65). NPs have been shown to be definitely an effective antibiotic against *E. coli*, *S. typhi*, *Staphylococcus epidermidis* and *S. aureus* (52). Increasing scientific evidence has demonstrated that AgNP activity would depend not only on their concentration and size (16,41), but also on their shape (45). In this regard, *E. coli*

seems to respond better to triangular nanoparticles and is inhibited at low concentrations (46). Pal *et al.*, 2014(35) studied the effect of nanoparticles with spherical, rod-like and triangular shapes against *E. coli*, they showed that all of them had antimicrobial activity, wihile the triangular nanoparticles being qualitatively more effective. Probably the triangular shape gives a greater positive charge to the nanoparticles, which together with the active facets on a triangular-shaped particle is able to ensure a greater activity.

It has been suggested that NPs also interfere with bacterial replication processes by adhering to their nucleic acids (41). This assumption, however, is controversial: for some authors NPs do not damage DNA (55), while according to others they intercalate into the DNA56). All factors which influence the activity of NPs(concentration, size, shape, UV radiation and the combination with various antibiotics) should be taken into consideration when preparing NPs for clinical use (20). Resistance to silver compounds as determined by bacterial plasmids and genes has been defined by molecular genetics, these findings should eliminate recent skepticism about the existence of silver-resistant bacteria. Now that the means for identifying silver resistance determinants in Enterobacteriaceae is available, similar efforts are needed with other common pathogens. The wide and uncontrolled use of silver products may result in more bacteria developing resistance(58). Notwithstanding the many conflicts in the literature regarding the effects of antibacterial NPs, it is likely that the result of a combined effect of each contributing feature, which provide a broad spectrum of antibacterial activity and decrease the probability of developing resistance (59). In Figure 2, the hypothesized bactericidal mechanisms are reported, where DNA damage through ROS is of particular interest and could be induced by NPs. In light of the decreasing effectiveness of classical antibiotics due to the emergence of biological resistance, the use of NPs in association with antibiotic drugs can be seen as an alternative for such difficult treatments(63). In fact, Singh *et al.*,2013(62) investigated individual and combined effects of NPs with 14 antibiotics belonging to seven classes against seven pathogenic bacteria using the disc-diffusion method. Their results showed the feasibility of the strategy, but different levels of activity increments, according to the class of antibiotic used, were observed. Aminoglycosides showed a small increase with the exception of gentamicin against *Acinetobacter baumannii* and kanamycin against *P. aeruginosa* (63). Considerable enhancement of the antibacterial effect was observed for amoxicillin in the presence of NPs against *P. aeruginosa* and penicillin demonstrated a 3-fold increase of efficiency against *Streptococcus mutans*. Vancomycin, with a 3.8-fold increase of activity against *Enterobacter aerogenes*, was reported to have the highest overall synergistic activity in combination with NPs compared to all other antibiotics(64). They also tested clinical derived bacterial strains, exhibiting resistance to one or more antibiotics belonging to the β-lactam class, and showed that the addition of NPs downsized MIC (minimum inhibitory concentration) into the susceptibility range, therefore, addition of NPs not only reduced MICs, but also rendered bacteria susceptible to antibiotic treatment(66,67).

This is of great importance since the administration of small amounts of NPs in combination with antibiotics can reduce the required dose of antibiotics to achieve the same effect by up to 1000-fold(64). Synergistic action of NPs and antibiotics

resulted in enhanced antibacterial effects; therefore, the simultaneous action of antibiotics and NPs can hamper the resistance development by pathogenic bacteria, also in view of the reduced amount of antibiotic administered (62,68). Fayaz *et al.*, 2010 (63) suggested that the increase in synergistic effect may be caused by the bonding reaction between antibiotic and NPs. They tested a set of antibiotics and found that the highest percentage of fold increase was obtained with ampicillin followed by kanamycin, erythromycin and chloramphenicol against all test strains. Interestingly, they realised that the percentage of fold increase in ampicillin with NPs against Gram-positive and Gram-negative bacteria were almost identical, even though inhibition of Gram-positive bacteria is generally more difficult to obtain with NPs alone. Furthermore, a different study analysed a set of clinical bacterial isolates exhibiting resistance against conventional sulphonamide (trimethoprim) andglycopeptides (vancomycin) antibiotics (64). A synergistic effect of antibiotics in conjugation with biologically synthesized NPs increased the susceptibility among the tested bacteria from 20% to 30%.The combined effect of NPs and antibiotics was notably against *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumonia*, *Bacillus* spp, and *Micrococcus luteus*. These results are also in line with the findings reported by Birla *et al.* 2009 (65) who registered increasing efficiency of antibiotics like vancomycin, gentamycin, streptomycin, ampicillin and kanamycin when used in combination with NPs against *P. aeruginosa*, *S. aureus* and *E. coli*.

Since NPs modified with different coatings such as polyethyleneimines (66), chitosan (48), glucosamine (67) and peptides (personal unpublished data)generally showed an increased antibacterial activity that has been related to the increased uptake as a consequence of a greater binding ability of nanoparticles to bacterial cells, Brown *et al.* (68) functionalised the surface of NPs with ampicillin (NP-AMP). They observed that NP-AMPs had increased biocidal activity compared to NPs. Their data suggested that the antimicrobial activity of functionalized NP-AMPs reside in the combined effect of the AgNP and the ampicillin carried onthe surface of the nanoparticle. The use of combination strategies for combating antibiotic resistance is slowly finding its way as a promising attempt to reduce the amount of antibiotics to be administered, therefore lowering the chances of steady resistance development. Selected studies on the antibacterial activity of NPsare summarized inTable 1.

The Antibiofilm Activity of Nanoparticles

Microorganisms growing in biofilms cause many of infections. The most common biofilm-forming bacteria associated with human infections are: *E. faecalis*, *S. aureus*, *S. epidermidis*, *Streptococcus viridans*, *E. coli*, *K. pneumoniae*, *Moraxella catarrhalis*, *Proteus mirabilis* and *P. aeruginosa* (15). Biofilms may be one of the leading causes for a shift from acute-phase diseases to chronic diseases. Most common diseases involving bacteria able to form biofilmare biliary tract infections, cystic fibrosis, dental caries, endocarditis, otitis and periodontal diseases(76).Moreover, several infections may be associated with foreign body material such as contact lens, sutures, artificial heart valves, arteriovenous shunts, catheters and orthopedic prostheses. The sites of infections may be different but the characteristics (mechanism for biofilm formation and development of resistance) of the causative agent are similar(16,17).

Biofilms are communities of

microorganisms attached to a solid surface, these adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance. Biofilm extracellular polymeric substance is a conglomeration composed of DNA, proteins and polysaccharides (73). The matrix is produced under the control of enzymes secreted in response to nutrient availability (74). Biofilms develop in natural aquatic systems, water pipes, on the teeth, on medical devices (15). The signals that promote biofilm rapid formation are:(i) presence of a suitable surface; (ii) increase of extracellular iron; (iii) presence of indole, polyamines, calcium and bile salts (75–77). In the initial phase of biofilm formation, bacterial attacks proliferate, forming microcolonies and attracting surrounding cells. The mature biofilm is a real microbial community that exchanges and shares products in a dynamic manner (78). In fact, cell growth, death, nutrients acquisition, the accumulation of waste products, mechanisms of motility and exopolysaccharide synthesis can affect the structure and attributes of biofilms (77,79).

A biofilm formation divided into the following phases: (i) the planktonic form, in which the separated cells are floating or swimming independently in a liquid support; (ii) the aggregated state, or sessile, in which cells are closely bound and firmly attached to one another and also, usually, to a solid surface. The change in behavior is triggered by a chemical communication mechanism that differs between species. Some species, for example, can produce acylhomoserine lactones as a "rest" signal, which induces planktonic cells that surround the phenotypic variation to change into the sessile state, through a different expression of the genes of the cell. As the understanding of biofilm increases, it is becoming evident that biofilm phenotypes cannot be analysed and eventually fought using the traditional principles of bacteriology. In fact, the properties of a biofilm are similar to the properties of a polymer and not to the properties of a sum of cells. Indeed, biofilms possess elastic and viscous properties which allows the community to adhere, grow in a tridimensional structure and move inside the lumen of a catheter or a similar device.

The pathogenicity of biofilms can be summarised by the following properties: (i) attachment to solid surfaces to high density; (ii) increased metabolic efficiency of the community; (iii)evasion of host-defences; (iv) horizontal gene transfer; (v) antimicrobial resistance; (vi) detachment of microbial aggregates able to colonise other sites (16,85). Bacterial biofilms are particularly unmanageable by antibiotic treatments not only due to an increase in transmission of resistance markers within the biofilm community, but also because the extracellular matrix hampers antibiotic diffusion, because the effectiveness of antibiotics is inactivated more easily, and because metabolically inactive persistent cells survive treatment. Together these features make bacterial biofilms up to 1000 times more resistant to antibiotics than planktonic cells (86,88). The antibiofilm activity of NPs has been demonstrated in a number of studies and is briefly described in the rest of the section (89,92,100). One pioneering study was performed to analyse the interactions of NPs with *Pseudomonas putida* biofilms. The results suggested that biofilms are impacted by the treatment with NPs. The nanoparticles analyzed in the study were ofquite large dimensions (over 60 nm) (80). One of the first reports on the antibiotic effect of NPs on *P. aeruginosa* and *S. epidermidis*, and their effect on biofilm formation, was produced by Kalishwaralal *et al.2010.*,(81). The study

focused on two important pathogens causing keratitis and the effect of a 2 h of treatment with NPs at a concentration of 100 μM showed that a 95% and 98% decrease of the biofilm was obtained. Therefore the authors concluded that NPs are able to induce the detachment of *P. aeruginosa* and *S. epidermidis* with rapidity and efficiency, opening clinical possibilities of alternative therapies(53).

An important feature to evaluate the real efficiency of the nanoparticles is derived from the chosen stabilization method employed. To this regard several coatings and chemicals have been reported:(i) starch was successfully employed to prepare NPs which had a disrupting effect on biofilms produced by *P. aeruginosa* and *S. aureus* (82); (ii) citrate-capped NPs of various sizes were shown to inhibit *P. aeruginosa*PAO1 biofilms (83); (iii) polyvinylpyrrolidone (PVP) showed good antibacterial activity towards *S. aureus*, *E. coli*, *P. aeruginosa*, *Bacillus subtilis*, and good fungicidal activity against various yeasts and molds (84); (iv) β-cyclodextrin is also an effective capping and stabilizing agent that reducesthe toxicity of NPs against the mammalian cell while enhancing their antibiofilm activity (85). Mohanty *et al,2012*(82) used a simple and environment friendly approach to form stable colloids of nontoxic NPs using starch to reduce metal nitrate to metal and simultaneously stabilize the nanoparticles in starch solution. Then they tested the effect of NPs on biofilm formation by *P. aeruginosa* and *S.aureus* with varying concentrations of NPs. Longer treatments (48 h) increased the antibiofilm efficiency to approximately 65% and 88% reduction in biofilm formation at micromolar concentrations. The ability to disrupt *P. aeruginosa* biofilm formation after treatment with the antimicrobial peptideLL-37, already known to impair biofilm formation, and NPs was also analysed and, in comparison toLL-37, treatment with NPs resulted in a 3-fold reduction of biofilm formation(90).

Multi-drug resistant (MDR) strains of *P. aeruginosa* were treated with NPs to investigate the eventual increased resistance compared to sensible strains. In the multidrug resistant strains, the inhibition rate of NPs was highest at concentration of 20 μg/mL similarly to the parental strain, therefore biofilms derived from multiresistant bacteria do no show an increased resistance to metal (69). Antibiofilm action of NPs of 8.3 nm in diameter stabilized by hydrolyzed casein peptides on Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *Serratia proteamaculans*) was investigated byRadzig*et al.2013*(86). A strong inhibition of biofilms formation was observed. Interestingly, several *E. coli* strains with mutations in genes responsible for the repair of DNA containing oxidative lesions (mutY, mutS, mutM, mutT, nth) were also analyzed and found less resistant to NPs than wild type strains, suggesting a possible involvement of these genes in repair of AgNP-produced damages to cellular DNA. The outer membrane of Gram-negative contains water-filled channels (called porins) to allow the exchange with the environment of low-molecular weight compounds. Porins are involved in the transport of Ag-ions and *E. coli* bacteria expressing mutated porin proteins are less susceptible to metal ions action (87). Radzig *et al.2013*,(86) confirmed that *E. coli* mutant strains deficient in OmpF or OmpC porins were 4–8 times more resistant to NPs when compared to the wild type strain, suggesting that porins have a key role in allowing NPs to exert their antibacterial effect.

The anti-biofilm activity of metal

nanoparticles was also demonstrated in other studies mainly focused on bacteria showing resistance to conventional antibiotics (88,89). The biofilm formation by methicillin resistant *S. aureus* (MRSA) and methicillin resistant *S. epidermidis* (MRSE) isolated from infected wounds was also analyzed by confocal laser scanning microscopy (CLSM) techniques which provided concrete evidence of the ability of NPs to block bacterial growth and to prevent the glycocalyx formation. A complete anti-biofilm activity was obtained with NPs at a concentration lower than 50 µg/mL (88). Gurunathan *et al.,2014*(90) analyzed the anti- bacterial and antibiofilm activity of antibiotics or NPs, or combinations of both against *P. aeruginosa*, *Shigella flexneri*, *S. aureus*, and *Streptococcus pneumonia*. They were able to show a clear enhancing effect for ampicillin andvancomycin against either Gram-negative or Gram-positive bacteria, suggesting that combining NPs withantibiotics could be a possible alternative therapeutic strategy against bacterial infectious diseases.

Table.1 Selected Studies on the Antibacterial Activity of Metal Nanoparticles (Sebasten Et Al,Gbj 2014)

Organism	Functionalization	Size (nm)	Effect	Ref.
E. coli *S. aureus*	unfunctionalized	Not declared	MIC 100 µg/mL	[4]
E. coli *S. typhi* *S. aureus*	unfunctionalized	10–15	MIC 25 µg/mL MIC 25 µg/mL MIC 100 µg/mL	[36]
E. coli	unfunctionalized	12	MIC_{70} 10 µg/mL	[32]
E. coli *S. aureus*	Unfunctionalized	13.5	MIC 3.3–6.6 nM MIC > 33 nM	[34]
P. aeruginosa	unfunctionalized	20–30	MIC 20 µg/mL	[69]
E. coli *V. cholerae* *S. typhi* *P. aeruginosa*	unfunctionalized	21	MIC 75 µg/mL	[33]
E. coli *S. aureus*	poly(amidehydroxyurethane)-coated	23	MIC 10 µg/mL	[37]
Brucella abortus	unfunctionalized	3–18	MIC 6–8 ppm	[70]
E. coli	citrate	30	MIC 5–10 µg/mL	[38]
S. aureus	unfunctionalized	5.5	MIC 0.2–4 µg/mL	[71]
E. coli	unfunctionalized	50	MIC_{99} 0.1 µg/mL	[35]
E. coli *S. aureus*	unfunctionalized	55	MIC 0.25 µg/mL	[40]
V. cholerae ETEC	unfunctionalized	88–100	MIC 1.6×10^3 for mL MIC 1.2×10^6 for mL	[72]

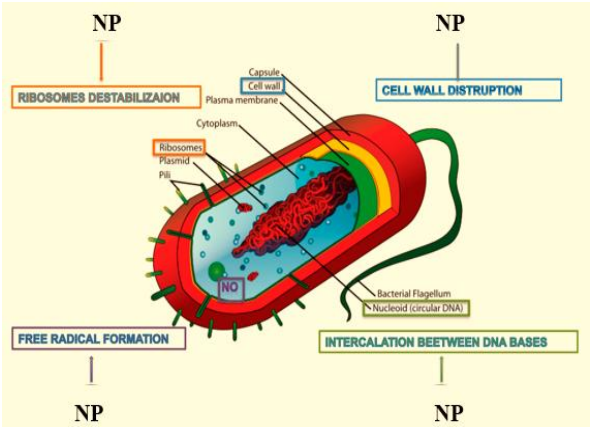

Figure.1 Mechanisms of Nps' Toxic Action (Mohammed et al., tib j 2012)

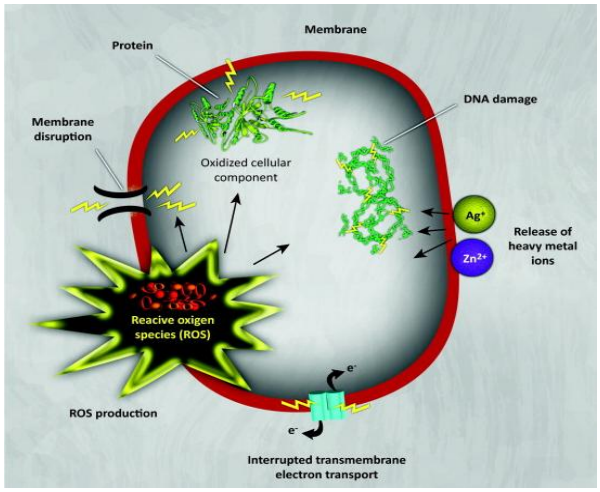

Figure.2 Mechanisms of Toxicity of Nanoparticles (nps) against Bacteria. Nps and their Ions (e.g., silver and zinc) can Produce Free Radicals, Resulting in Induction of Oxidative Stress (i.e., Reactive Oxygen species; ros). The produced Ros can Irreversibly Damage Bacteria Resulting in Bacterial Death (Trends in Biotechnology, January 2013).

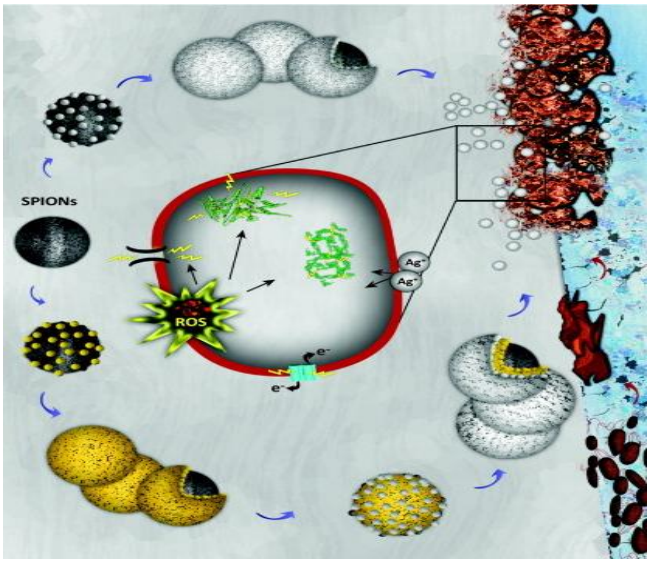

Figure.3 Schematic representation of toxicology effect of multifunctional nanoparticles (NPs) in bacterial biofilms. Monodisperse superparamagnetic iron oxide NPs (SPIONs; black spheres) are coated with silver (gray shell), gold (yellow shell), and silver ring-coated, gold-coated SPIONs; silver ring-coated SPIONs and silver ring-coated, gold-coated SPIONs have strong toxic effects on bacterial biofilms, by penetration into the biofilms. Both SPIONs cores and the intermediate gold shell have the capability to induce heat by applying alternative magnetic and laser fields, respectively; the produced heat can be used as additional means to escalate bacterial death using these NPs. The magnified section in the center illustrates the irreversible effects of NPs and their ions on the various parts of the bacteria (Trends in Biotechnology, January 2013)**.**

An interesting evolution of using nanoparticles against bacterial biofilms is represented by metal-coated magnetic nanoparticles, in fact, engineered multimodal nanoparticles comprising amagnetic core and a metal ring showed promising results (91). Along this line, magnetic and antibacterial properties have been exploited by creating super paramagnetic iron oxide nanoparticles (SPION)conjugated with metal to demonstrate that MRSA biofilms can be eradicated without the need of antibiotics(88).MRSA biofilms treated with 1 mg/mL of metal-conjugated SPION resulted in a consistent mass decrease (11,53,17) as shown in figure 3.Moreover, SPION anti-biofilm efficacy is further improved in the presence of an external magnetic field. Metalis nowadays used on medical devices to support anti-biofilm activity. Biofilms from clinical isolates of *P. aeruginosa* were treated with gum arabic capped-metal nanoparticles (GA-NPs) showing a concentration dependent inhibition of bacterial growth and treatment of catheters with GA-NPs at50 µg/mL resulted in 95% inhibition of bacterial colonization of the plastic catheter surface (88).Other authors have also shown the applications of nanometal as antibiofilms for coating catheters with positive results against both Gram-positive and Gram-negative bacteria(92,94). Furthermore, no significant accumulation of metal was detected in the main organs of the test animals in which engineered catheters had been implanted (92).

Dental applications were obtained with composites containing metal nanoparticles that can act against *S. mutans* biofilm (95). Also, bone cements modified with NPs significantly reduced biofilm formation on the surface of the cement (96). Some medical devices, as well as surgical masks (97), coated with NPs are already in clinical trials with promising results (93,98,99). Furthermore, recent studies suggest the use of wound dressings treated with NPs to prevent or reduce microbial growth in wounds and to improve the outcomes of healing (100). A bioactive chitosan hydrogel membrane including NPs showed a synergistic activity of chitosan and NPs to reduce the growth of *S. aureus*, *E. coli*, *S. epidermidis*, *P. aeruginosa* strains and to disrupt mature biofilms (101).

In conclusion, antibacterial activities of NPs depend on two main factors:(i) physicochemical properties of NPs and (ii) type of bacteria, it is now clear that NPs possess a strong antibacterial activity highlighted by several studies. NPs have the ability to interact with various microorganisms (such as bacteria) and also impact both the growth and mature bacterial biofilms, and therefore, could be used as broad spectrum antimicrobials to control infection. The antibacterial effect appears to be conferred by their ultra small size and increased surface area, through which they destroy the membrane, cross the body of the microbe and create intracellular damage. Due to the structural difference in the composition of the cell walls ofGram-positive and Gram-negative NPs have significantly less effect on the growth of Gram-positive bacteria, the Gram-negative bacteria have a layer of lipopolysaccharides on the outside, and present below a thin (7 to 8 nanometers) layer of peptidoglycan. Although lipopolysaccharides are composed of lipids covalently bound to polysaccharides, there is a lack of rigidity of the overall structural envelope. The negative charges on the lipopolysaccharides are attracted to the weak positive charge of NPs, on the other hand, the cell wall of Gram-positive bacteria is mainly composed of a thick layer(20 to 80 nanometers) of

peptidoglycan consisting of linear polysaccharidic chains cross-linked by short peptides to form a three-dimensional rigid structure. The stiffness and the extensive cross-linking not only reduce the bacterial cell wall anchoring sites for NPs but also render the wall itself more difficult to penetrate.

However, the same features that make NPs attractive, at the same time raise important issues such as the toxicity and environmental safety. NPs' antibacterial effects have been described in detail, but their mechanism of action is still unclear. A multifaceted mechanism against microorganisms seem to be due to nanoparticle interactions with the bacterial surfaces, as well as to their particular structure. Defining NPs' mechanism of action is, nowadays, a priority for biomedical research and more research on the bioactivity and biocompatibility of NPs is necessary. Understanding the kinetics of dissolution that lead to transformations of NPs in the presence of specific inorganic ligands is crucial to determining their antimicrobial activity and overall toxicity in the environment. Metal ions (Ag+), released by NPs, are likely to interact with chloride (Cl−) which is often present in bacterial growth media and exhibits a strong affinity for oxidized metal. High concentrations of chloride ions in the routinely used media can cause precipitation of Ag ions as AgCl, thus masking the contribution of dissolved metal to NPs antibacterial effect. This consideration should influence the choice of the medium to be used when evaluating antimicrobial effects and more studies are needed to investigate the contribution of AgCl to the observed antibacterial activity of NPs.

The studies on the combined use of NPs with other antimicrobial agents can help reduce the problem of toxicity and to avoid the potential for development of resistance, and above all strongly enhance the microbicidal effect. The broad spectrum of bioactivity of NPs makes them promising agents not only to fight infections, but in many other biomedical areas.

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